CERTAIN INTERRELATIONSHIPS IN THE METABOLISM OF GLYCINE AND SERINE*

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The utilization of L-serine-2,3-deuterio,3-C14,N15 and glycine-2-C14,N15 for the biosynthesis of purines, thymine, and choline in the rat was described briefly in preliminary publications from this laboratory (1, 2). In the synthesis of the methyl groups of choline from the β-C14DaOH group of isotopically labeled serine it was later found (3) that the C14:D ratio was unaltered, indicating that carbon-hydrogen bonds were not broken. In order to gain a better understanding of the reactions involved in these conversions a more careful investigation was carried out, in the same animals, of the interrelationships in the metabolism of glycine and serine.

EXPERIMENTAL

Labeled Compounds1—The labeled L-serine was the same as that previously described (4). It had 3.28 × 10^6 c.p.m. in the β-carbon atom, 25.8 atom per cent excess N15, and 18.5 atom per cent excess deuterium. In order to determine the deuterium distribution in the molecule, 40.2 mg. of the labeled compound were diluted with 261.0 mg. of normal serine and degraded by the procedure described later. The deuterium concentration on the β-carbon was found to be 35.5 atom per cent excess. This would indicate that 45 per cent of the deuterium was in the α position and 55 in the β position. It can be calculated that the deuterium concentration on the α-carbon atom was 58.0 atom per cent excess.

The labeled glycine in one experiment was a mixture of glycine-N15 and glycine-2-C14. The N15 concentration was 20.6 atom per cent excess and the activity 3.44 × 10^6 c.p.m. in the α-carbon atom. In a second experi-

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1 C14 was obtained on allocation from the United States Atomic Energy Commission.
ment glycine-2-C\textsuperscript{14}, having an activity of 2.23 \times 10^6 c.p.m. in the \(\alpha\)-carbon, was used without the N\textsuperscript{15} label.

**Feeding Experiments**—Four male Sherman rats in individual metabolism cages were accustomed to a 15 per cent casein diet (5) for a few days. The serine was incorporated at a level of 0.47 mM per 100 gm. of body weight into 8 to 10 gm. of ration per rat per day for 2 days. The glycine-2-C\textsuperscript{14}, N\textsuperscript{15} was similarly administered at 0.40 mM per 100 gm. of body weight in 8 to 10 gm. of ration per rat for 1 day. Glycine-2-C\textsuperscript{14} (0.426 mM per 100 gm. of body weight per day) was fed to 1 rat for 3 days. The results of this experiment (6), which agreed very closely with those of the 1 day feeding, are reported in parentheses in the fourth column of Table II. When \(\alpha\)-amino-\(\gamma\)-phenylbutyric acid was administered, it was added to the diet, 1 mg. per gm. of body weight being used.

**Isolations**—The internal organs (liver, heart, kidneys, lungs, spleen, small intestine, testes) were homogenized in alcohol, extracted twice with boiling absolute alcohol (8 hours) and boiling 1:1 alcohol-ether (16 hours), and dried in a desiccator. Nucleic acids were extracted with hot 10 per cent NaCl solution and the tissues treated with 6 per cent trichloroacetic acid. The insoluble residue was hydrolyzed with 20 per cent HCl and the hydrolysate distilled in vacuo to remove HCl.

The residue was taken up in a small volume of water and the solution passed through a column of Duolite A-4 to retain glutamic and aspartic acids. The neutral and basic amino acids were eluted with water, and the acidic amino acids with 1 N HCl. After concentration to a small volume, the aqueous eluates were passed through a column of Amberlite IRC-50 (at pH 4.7 with acetate buffer) to remove basic amino acids. The neutral fraction was eluted with water, concentrated to a small volume, and placed on a column of Dowex 50 (600 \times 7.5 mm.) for isolation of serine, alanine, and glycine (7, 8) by elution with 1.5 N HCl with use of an automatic fraction collector. The composition of every fifth fraction (approximately 20 ml.) was estimated by one-dimensional paper chromatography with aqueous phenol in an atmosphere of ammonia (9).

Serine and threonine came off the column together. With the aid of a mixture of L-serine and L-threonine-4-C\textsuperscript{14} (10) it could be demonstrated that pure serine is obtainable from this solution by precipitating it as the \(p\)-hydroxyazobenzene-\(p\)'-sulfonate (11) and recrystallizing the salt twice from water at \(-5^\circ\). Serine \(p\)-hydroxyazobenzene-\(p\)'-sulfonate, C\textsubscript{16}H\textsubscript{17}O\textsubscript{7}N\textsubscript{3}S \cdot H\textsubscript{2}O (401.4), calculated N 10.5; found N 10.3. Serine, C\textsubscript{3}H\textsubscript{7}N\textsubscript{2}O\textsubscript{3} (105.1), calculated N 13.3; found N 13.1.

Glycine and alanine overlapped to some extent, but sufficient quantities were obtained from the uncontaminated fractions for recrystallization to
constant activity prior to degradation. Alanine, C₃H₇NO₂ (89.1), calculated N 15.7; found N 15.2. Glycine, C₂H₅NO₂ (75.1), calculated N 18.7; found N 18.4.

Acetyl-α-amino-γ-phenylbutyric acid was isolated in the usual manner, hydrolyzed, and converted to the silver acetate.

Degradation Procedures—Serine, obtained by decomposition of the p-hydroxyazobenzene sulfonate with barium acetate (11), was degraded with periodate according to known procedures (12), except that formaldehyde, representing the β-carbon atom, was obtained as the dimedon derivative and used as such for the determination of C¹⁴ activity and deuterium content. The difference between the deuterium concentrations of the serine and β-carbon represents that of the α position. The N¹⁵ of serine was determined on the ammonia remaining from the periodate oxidations, which was aspirated out of the alkaline reaction mixture after the addition of Zn powder.

Alanine was degraded with ninhydrin to CO₂, NH₃, and acetaldehyde which was further degraded with hypoiodite (13).

Glycine was decomposed by ninhydrin at pH 2.5 to CO₂ (which was obtained as BaCO₃), formaldehyde, which was distilled out of the reaction mixture and counted as the dimedon derivative, and ammonia (13).

Silver acetate was degraded with bromine in carbon tetrachloride (14, 15) and the resulting CO₂ precipitated as BaCO₃.

Isotope Analyses—C¹⁴ activity was measured with a thin window, or an internal flow, Geiger-Müller counter either on the compounds isolated or, when CO₂ was isolated, on BaCO₃. Stainless steel dishes (2.0 sq. cm. in area) were used, and, nearly always, sufficient sample was taken to give infinite thickness. When necessary, corrections to infinite thickness or for back-scattering were applied. Sufficient counts were taken to give a standard deviation of less than 5 per cent. The values in Tables I and II (counts per minute per dish of carbon) were obtained from the observed counts by dividing by the fraction of carbon in the compound. When the position of the label was known, the observed count was divided by the fraction of labeled carbon in the compound to yield counts per minute per dish of labeled carbon.

N¹⁵ (16) and deuterium (17) were determined by standard procedures.

RESULTS AND DISCUSSION

Utilization of L-Serine-2,3-D,3-C¹⁴, N¹⁵ and of Glycine-2-C¹⁴, N¹⁵ for Serine and Glycine of Internal Organs—The data in Tables I and II describe the manner in which dietary serine and glycine are utilized for a few constituents of internal organ proteins. In order to facilitate comparison be-
between the two experiments the results are also expressed as coefficients of utilization\(^2\) (18) which reduce the observed isotope values to the same level of activity and dose in the various precursors.

The highest isotope concentrations are found, as expected, in the amino acid administered. Aside from the labilization of deuterium in the \(\alpha\) position, the apparent stability of internal organ serine is indicated by coefficients of utilization of 73, 72, and 77 for the \(\beta-C^{14}, \beta-D,\) and \(N^{15}\), respectively (Table I). The \(\alpha\)-hydrogen of glycine is also known to be labilized

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\text{Table I}
\]

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<th>Compound isolated or position in molecule</th>
<th>Isotope concentration</th>
<th>Coefficient of utilization</th>
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<tr>
<td></td>
<td>(C^{14})</td>
<td>(D)</td>
</tr>
<tr>
<td></td>
<td>atom per cent excess</td>
<td>atom per cent excess</td>
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<tr>
<td>Serine</td>
<td>7,080</td>
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<td>Carboxyl</td>
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<td>(\alpha)</td>
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<td>(\beta)</td>
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<td>Methyl</td>
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* Fed to rats 0.47 mm per 100 gm. per day for 2 days. Activity \(3.28 \times 10^4\) c.p.m. per dish of labeled carbon under standard conditions; \(D\) 58 and 35.5 atom per cent excess in the \(\alpha\) and \(\beta\) positions, respectively; \(N^{15}\) 25.8 atom per cent excess.

† Amino acids from proteins of internal organs; acetate from acetylphenylaminobutyric acid.

\(^\$\) Counts per minute per dish of labeled carbon.

\(^\$\) Calculated value; see the experimental part.

The coefficient of utilization (18) is derived from the isotope dilution formula (19) \(b = a(x/y - 1)\), where \(a =\) millimoles of labeled compound administered per 100 gm. per day, \(b =\) millimoles of material per 100 gm. per day elaborated by the organism and used to dilute \(a, x =\) isotope concentration of \(a,\) and \(y =\) isotope concentration of the compound isolated. The coefficient of utilization = \(1000/b = 1000/a(x/y - 1).\) It is expressed as 1000 times the reciprocal of \(b\) so that it will show an increase with increasing utilization. As modified for use with isolated tissue constituents, \(a\) becomes the total rather than the daily dose.
in vivo (20), and this is further illustrated by nearly complete loss of deuterium from the glycine derived from 2,3-deuterioserine, although the N\textsuperscript{15} concentration of the glycine is 63 per cent of that of the internal organ serine.

A similar situation occurs when glycine-2-C\textsuperscript{14},N\textsuperscript{15} is administered (Table II). The N\textsuperscript{15} and C\textsuperscript{14} levels in the serine of the internal organs are 73 and 64 per cent, respectively, of those of the glycine. The labilization of the \(\alpha\)-hydrogen atom of serine, discussed previously, could be due to resynthesis from glycine which has undergone this labilization. The glycine of the

<table>
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<th>Coefficient of utilization</th>
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</thead>
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<tr>
<td></td>
<td>C\textsuperscript{14}</td>
<td>N\textsuperscript{15}</td>
</tr>
<tr>
<td></td>
<td>c.p.m. (\times 10^3)</td>
<td>atoms per cent excess</td>
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<td>Glycine</td>
<td>35.8</td>
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<td>1.19</td>
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<tr>
<td>(\beta)</td>
<td>0.845</td>
<td>0.9 (0.8)</td>
</tr>
</tbody>
</table>

\* Fed to rats 0.40 mm per 100 gm. for 1 day. Activity 3.42 \(\times 10^4\) c.p.m. per dish of labeled carbon under standard conditions; N\textsuperscript{15} 20.6 atom per cent excess.

\dagger Amino acids from proteins of internal organs.

\ddagger The figures in parentheses are duplicate results obtained in an experiment with glycine-2-C\textsuperscript{14} (6). See the experimental part for details.

\$ Counts per minute per dish of carbon.

internal organs has nearly the same ratio of C\textsuperscript{14}:N\textsuperscript{15} as the dietary glycine, with a slightly greater utilization of the N\textsuperscript{15} than of the C\textsuperscript{14}. This trend is further accentuated in the serine, in which the utilization of N\textsuperscript{15} for the amino group is about 20 per cent higher than that of the C\textsuperscript{14} for the \(\alpha\)-carbon; this may be another illustration of the effective acceptance of nitrogen by endogenous precursors of the carbon skeleton of glycine (21, 22).

Serine and glycine, together with aspartate and glutamate, are probably the most metabolically active amino acids. In contrast to aspartate and glutamate, which play a central rôle in nitrogen transfer reactions, serine and glycine have remarkably stable carbon-nitrogen bonds. Some of the
major reactions of these amino acids involve a part or all of the carbon chain accompanied by the nitrogen.

It is yet to be explained, however, how the ratio of $^{14}C: N^{15}$ in $\beta-C^{14}, N^{15}$-labeled serine remains unchanged despite the rapid and reversible cleavage of serine to glycine and a 1-carbon fragment. This reaction, which is known from other work (23–25), is further illustrated in Tables I and II. Since at least two-thirds of the serine appears to be derived from glycine, and since after administration of labeled serine the concentration of $N^{15}$ in the glycine is nearly two-thirds that of the serine from the same internal organs, maintenance of an unaltered $C^{14}: N^{15}$ ratio would require that roughly as much $C^{14}$ enters the serine from the 1-carbon pool as $N^{15}$ from glycine. This would suggest that the amount and rate of turnover of 1-carbon compounds and of glycine are approximately equal. The unchanged $\beta-C^{14}: N^{15}$ ratio found in serine is, therefore, the result of compensating reactions rather than of the biochemical stability of dietary serine. The fact that the $\beta-C^{14}: \beta-D$ ratio is also unaltered would imply that the level of oxidation of the $C_1$ compound is not raised during the resynthesis of serine from labeled fragments.

Formation of Alanine and Acetate from Glycine and Serine—In two separate experiments the ratio of activities of the $\alpha$-carbon to that of the $\beta$-carbon of serine after the administration of a relatively small quantity (0.4 mm per 100 gm.) of glycine-2-$C^{14}$ was 3. This is higher than the ratio observed when much larger quantities of labeled glycine were administered (24). The acetate from acetylphenylaminobutyric acid obtained in one of these experiments (6) was found to be nearly equally labeled in both atoms. The results shown in Table II indicate that equalization of label takes place through intermediates of the tricarboxylic acid cycle. The ratio of activities $\alpha-C^{14}: \beta-C^{14}$ found in the alanine is 1.4, which suggests that alanine and acetate are derived from glycine by way of serine (24) and pyruvate (26). A similar sequence of reactions may be operative in the fermentation of glycine to acetate by *Dylococcus glycinophilus* (27, 28).

We are indebted to Mr. I. Sucher for the $N^{15}$ and deuterium analyses, and to Mr. B. Gourdin for technical assistance.

**SUMMARY**

Certain aspects of the interconversion of serine and glycine were studied with the aid of L-serine-3-$C^{14}, 2,3-D, N^{15}$ and glycine-2-$C^{14}, N^{15}$.

**BIBLIOGRAPHY**


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